We wish to express our thanks to Mr T. C. Grey for skilled technical assistance and to the Medical Research Council for a research studentship (to J. B. C.) and a research expenses grant (to S. V. P.).

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Ultraviolet Fluorescence of the Aromatic Amino Acids

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(*Received* 25 *June* 1956)

Compounds resembling the aromatic amino acids are known to show appreciable fluorescence in the near ultraviolet [Ley & Englehardt (1910), Kowalski (1911), Marsh (1924)], but the fluorescence of the aromatic amino acids themselves has not so far been unequivocally characterized. Debye & Edwards (1952) have made observations of the phosphorescence of the aromatic amino acids. The position of the phosphorescence bands and the decay times found by these authors indicate the probable existence of fluorescence bands in the near ultraviolet with normal decay times (McClure, 1949).

It is shown in this paper that the three aromatic amino acids exhibit characteristic ultraviolet fluorescence. The fluorescence-excitation spectra, fluorescence spectra and quantum yields of aqueous solutions have been studied. Further papers will deal with aspects of the ultraviolet fluorescence of peptides and proteins.

Characterization of a substance as a fluorescent entity

It is often necessary to determine whether the fluorescence shown by a solution is due to a given substance present in it. To ascribe the observed fluorescence to a given component of the system the following criteria are proposed:

Fluorescence-excitation spectrum. The quantum yield of the fluorescence of a substance in *solution*, defined as the ratio of the number of quanta emitted to the number of quanta absorbed, is known to be independent of the exciting wavelength, at least for excitation with light in the air ultraviolet ($\lambda > 2000$Å) and visible regions of the spectrum (Wavilov, 1927; Neporent, 1947; Weber & Teale, in preparation). Therefore,

$$F(\lambda) = kqA(\lambda), \qquad (1)$$

in which $F(\lambda)$ is the fluorescence intensity set up by excitation with light of wavelength λ, $A(\lambda)$ the number of photons absorbed in the solution, q the quantum yield and k a constant depending on the general geometry of the system and the distribution of the intensity along the exciting beam. A solution of optical density $E(\lambda)$ illuminated with light of this wavelength of intensity $I(\lambda)$ photons absorbs

$$A(\lambda) = I(\lambda)\,(1 - 10^{-E(\lambda)}). \qquad (2)$$

From (1) and (2)

$$F(\lambda) = qkI(\lambda)\,(1 - 10^{-E(\lambda)}). \qquad (3)$$

For an arbitrary wavelength $\bar{\lambda}$

$$qk = F(\bar{\lambda})/I(\bar{\lambda})\ (1-10^{-E(\bar{\lambda})}). \qquad (4)$$

If (4) is used to eliminate qk from (3)

$$F(\lambda) = \frac{F(\bar{\lambda})\ I(\lambda)}{I(\bar{\lambda})}\left[\frac{1-10^{-E(\lambda)}}{1-10^{-E(\bar{\lambda})}}\right].$$

$\log \Omega$ may now be defined as

$$\log\Omega \equiv \log 1\Big/\left\{1-\frac{F(\lambda)/F(\bar{\lambda})}{I(\lambda)/I(\bar{\lambda})}\ (1-10^{-E(\bar{\lambda})})\right\} = E(\lambda). \qquad (5)$$

$\bar{\lambda}$ may be conveniently chosen to be the wavelength of the maximum of the absorption band of least frequency. Then $F(\lambda)\ I(\bar{\lambda})/F(\bar{\lambda})\ I(\lambda)$ is simply the fluorescence relative to that set up by excitation with the wavelength of the absorption maximum referred to equal photon excitation. Equation (5) shows that a plot of $\log \Omega$ against λ should reproduce the absorption spectrum of the fluorescence entity which may be identified in this way. It must be stressed that k is very sensitive to a change in the distribution of the fluorescent intensity along the exciting beam, and therefore equation (5) is only applicable when $E(\lambda)$ is small. In practice $E(\lambda)$ must be less than 0·1, preferably less than 0·05. When $E(\bar{\lambda})$ may be reduced to 0·05 or less equation (5) may be reduced to the simpler form

$$E(\bar{\lambda})\ \frac{F(\lambda)\ I(\bar{\lambda})}{F(\bar{\lambda})\ I(\lambda)} = E(\lambda). \qquad (6)$$

Fluorescence spectrum. It is known that the fluorescence spectrum continues the absorption spectrum towards longer wavelengths. Although the difference between the maximum of absorption λ_A and the maximum of emission λ_F varies considerably from one substance to another and often in the same substance depending on the solvent, always $\lambda_F > \lambda_A$. This provides a useful criterion to exclude certain components of a solution as responsible for the observed fluorescence.

Quantum yield. The possibility that the fluorescence of a solution may be due to an impurity can often be resolved by a knowledge of the overall quantum yield. In a system consisting of several components the overall quantum yield due to excitation by light of wavelength λ is

$$\overline{q(\lambda)} = q_a\frac{E_a}{E(\lambda)} + q_b\frac{E_b}{E(\lambda)} + \ldots + q_n\frac{E_n}{E(\lambda)},$$

where q_a, q_b, ..., are the quantum yields and $E_a/E(\lambda)$, $E_b/E(\lambda)$, ..., the fractional absorptions of the respective components. Let the principal component of the system be a. Since all q's $\leqslant 1$ and

$$E(\lambda) = E_a + E_b + \ldots + E_n,$$

$$\overline{q(\lambda)} \leqslant q_a\frac{E_a}{E(\lambda)} + \frac{E(\lambda)-E_a}{E(\lambda)};$$

but also $E_a/E\lambda \leqslant 1$. Therefore

$$\left.\begin{aligned}\overline{q(\lambda)} &\leqslant q_a + \left[1-\frac{E_a}{E(\lambda)}\right]\\ \text{and}\qquad q_a &\geqslant \overline{q(\lambda)} - \left[1-\frac{E_a}{E(\lambda)}\right].\end{aligned}\right\} \qquad (7)$$

Thus in stable substances purified by crystallization a quantum yield of a few per cent indicates that the fluorescence is due to the main component of the system.

EXPERIMENTAL

The fluorescence-excitation spectra were determined by means of the apparatus the block diagram of which is shown in Fig. 1. Light from a high-pressure Nester-type hydrogen arc H enters the monochromator after passing through the collimating system K. The monochromatic beam emerges from the exit slit X into the light-tight box B containing the cell C filled with the fluorescent solution. The cell axis makes an angle of 15° with the line of view of the detector so that reflected exciting light is thrown on to the blackened wall of the box opposite to the detector. This is a 27M3 Mazda photomultiplier. The envelope of this type of photomultiplier is transparent to wavelengths longer than 2200 Å. A stabilized power supply was used to energize the photomultiplier (Fellgett, 1954) and the photoelectric currents were read directly on a Scalamp galvanometer. The stability of the system permitted the determination of photoelectric currents to within $\pm 2\cdot10^{-3}$ μA. In solutions of amino acids the scattering of the exciting light was negligible and no filter was required to separate the fluorescent from the exciting light. Ordinary fused-quartz cells such as those used in ordinary spectrophotometric work were found to give a blue fluorescence when irradiated with light of wavelength 230–260 mμ. The response of the photomultiplier was thus a response to the fluorescence of both cell and solution. Although a correction for the cell fluorescence is simply done by determining the response to the cell and solvent on one hand and to the cell and solution on the other, it is important to keep this correction as small as possible. The fluorescence of cells made of Ultrasil Haereus

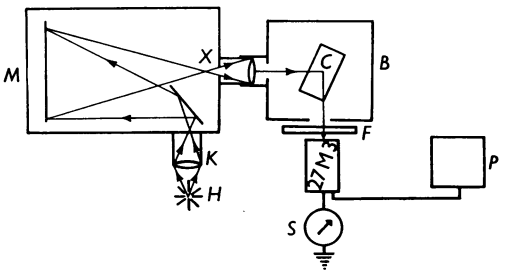

Fig. 1. Experimental arrangement for determination of excitation spectra of fluorescence. H, Nester-type hydrogen arc; K, collimating system; M, grating monochromator; X, exit slit; B, light-tight box containing the silica cell C with the fluorescence solution; F, filter to eliminate exciting light; 27M3, Mazda photomultiplier with P, power pack and S, Scalamp galvanometer.

(Quartz Glass Ltd., Barkingside, Essex) was found to be six to eight times weaker than that of cells of ordinary fused quartz. Such cells were used throughout this work. The monochromator was a Bausch & Lomb instrument with an aluminium grating blazed for the first order in the ultraviolet, giving a dispersion of 3·3 mμ./mm. at the exit slit. To determine excitation spectra the relative distribution of energy in the spectrum of the source was obtained by two methods. The first was the blackening of a photographic plate (e.g. Weissberger, 1946), the validity of the reciprocity law being assumed. The second method consisted in the use of a concentrated solution of a fluorescent substance as a proportional photon counter (Bowen, 1936; Bowen & Sawtell, 1937). As the fluorescent yield and spectrum are independent of the exciting wavelength, if the concentration of the solution is such that total absorption of the beam takes place in a very thin layer at the front of the cell, the dimensions of the fluorescent volume, and therefore the geometry of the system, remain approximately independent of wavelength and the response of the detector will be proportional to the number of photons absorbed by the solution. At the concentrations required for complete absorption of the light in a very thin layer ($>10^{-2}$ M) many fluorescent substances form an appreciable proportion of non-fluorescent dimers. As the absorption spectrum of the dimers differs from that of the monomers the fluorescent yield will be found to vary with wavelength. The selection of a suitable fluorescent substance for this purpose was therefore a matter of trial and error. Substances in which the fluorescent yield of the concentrated solution increases markedly with temperature or in which the absorption spectrum of the concentrated solutions shows absorption bands absent in the dilute solutions are therefore inadequate for this purpose (Förster, 1951). If water solutions were used, a relatively small molecular ion offered the best chances of remaining unaggregated at high concentrations. Solutions (10^{-2} M) of sodium 1-dimethylaminonaphthalene-5-sulphonate, 1-dimethylaminonaphthalene-7-sulphonate and 1-aminonaphthalene-3:6:8-trisulphonate in water were used. These substances have the further advantages that the overlap of the absorption and emission bands is negligible and that the quantum yields of the first two are very high (0·75 for 1-dimethylaminonaphthalene-7-sulphonate, 0·53 for the 1:5 isomer) (Weber & Teale, 1956).

Fig. 2 shows that the results obtained with these salts agreed very satisfactorily with the photographic method. Finally, if the direct response of the photomultiplier to the source was corrected, with the spectral-response curve provided by the manufacturers, good agreement with the other methods was obtained for the wavelengths to which the photomultiplier envelope was transparent ($\lambda > 2200$ Å).

Fluorescence spectra

These were determined with the monochromator as shown in Fig. 3. The cell C containing the fluorescent solution was mounted in front of the collimator K of the monochromator. The fluorescence was excited by light from the low-pressure mercury arc (Westinghouse Sterilamp 794) filtered through a 3 cm. layer of 0·01 M p-nitrophenol and M-$NiSO_4$ solution in water. This combination effectively isolated the 2537 Å line, which carried over 90% of the unfiltered energy output of the source from the other ultraviolet mercury lines. When very weak ultraviolet fluorescences were studied with this excitation filter the weak mercury lines at 2980, 3130 and 3350 Å were recorded and appeared superimposed on the fluorescent spectrum. Fortunately with the aromatic amino acids the fluorescence was sufficiently strong to make unnecessary any correction for the breakthrough of these lines. As shown in Fig. 3 the fluorescent light leaves the cell after traversing a variable thickness of solution where some of the fluorescent light may be reabsorbed to excite further fluorescence. The spectrum of the fluorescent light entering the monochromator is the technical spectrum (Birks, 1954) which differs from the molecular spectrum in two respects: the emission at wavelengths at which the absorption and fluorescence spectra overlap appreciably appears attenuated, and since the fluorescence spectrum is independent of the exciting wavelength, the re-emission of part of the absorbed light increases the emission at those wavelengths where overlap is negligible. Thus the corrections to be applied to obtain the molecular from the technical spectrum are an attenuation correction and a re-emission correction.

Attenuation correction. If $\alpha(\lambda)$ is the transmission coefficient of the solution for wavelength λ, $\bar{l}$ the average thickness of solution traversed by the rays entering the

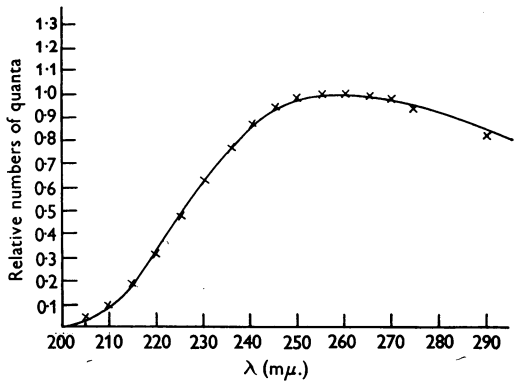

Fig. 2. Spectral photon distribution of the hydrogen arc, as emerging from grating monochromator. The continuous line is the plot of the distribution obtained by the use of fluorescent solutions of naphthylaminesulphonic acids as photon counters. The crosses are the values from the Ilford ultraviolet Q3 plates, using the reciprocity law.

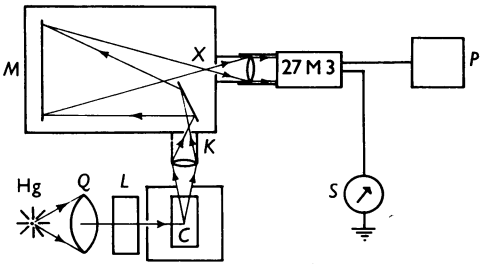

Fig. 3. Experimental arrangement for determination of fluorescence spectra. Hg, Low-pressure mercury arc (Sterilamp 974); Q, quartz lens; L, liquid filter (3 cm. layer of 0·01 M p-nitrophenol and M-$NiSO_4$); C, cell containing fluorescent solution; K, M, P, S and X as in Fig. 1.

monochromator and $F(\lambda)$ the intensity at the exit slit corrected for the change of response of the detector with wavelength and for losses in the grating, then $F(\lambda)/\alpha(\lambda)^{\bar{1}}$ is the intensity corrected for attenuation.

Re-emission correction. Although it is difficult to derive an exact expression for the re-emission correction, the order of this correction may be estimated as follows. The ratio of the areas under the emission bands with and without correction for attenuation is

$$\beta = \int F(\lambda)/\alpha(\lambda)^{\bar{1}}\,d\lambda \Big/ \int F(\lambda)\,d\lambda. \qquad (8)$$

The fractional contribution of the secondary fluorescence to the total fluorescence entering the monochromator is $(\beta - 1)\,q$, and this will be the fractional distortion of the band when the correction is ignored. If a sufficiently dilute solution is used $\beta - 1$ can be reduced to a few per cent. In such cases if q itself is small the re-emission correction may be altogether ignored as it falls within the experimental error of the determinations.

Correction for attenuation by the grating. The relative attenuation by the grating was determined as follows: light emerging at the exit slit of the grating monochromator was allowed to fall upon the entrance slit of a D. 246 Hilger prism monochromator, the detector being placed at the exit slit of the latter instrument. The band width of the light emerging from the grating instrument was kept large so that the effective band width of the light reaching the detector was always determined by the prism instrument. The source was then placed directly in front of the entrance slit of the prism instrument and the response of the detector to the range of wavelengths again determined. The ratio of the response to light reaching the detector through grating and prism to the response through prism alone is proportional to the grating transmission for this wavelength. The slit widths of both instruments were kept constant throughout the calibration. The relative attenuation by the grating was found to change slowly with wavelength, less than 0·5 %/mμ. in the region of most rapid change. Thus the distortion introduced by ignoring the grating correction is small for the relatively narrow fluorescent bands but becomes important in the determination of relative quantum yields, when the areas under the bands emitted at very different wavelengths have to be compared.

Quantum yields of fluorescence

The method of Weber & Teale (1956) was used to determine the quantum yield of the fluorescence of the amino acids. In this method the intensity of the fluorescence emitted at right angles to the direction of excitation is compared with the intensity of the light scattered in the same direction by a glycogen solution. If the exciting radiation is monochromatic and the apparent optical densities of the scattering and fluorescent solutions are the same, the glycogen solution acts as a standard of quantum yield 1. Then the ratio of the fluorescent to the scattered intensity is proportional to the quantum yield, after a correction for the unequal spatial distribution of the radiation has been applied. This correction may be computed from measurements of the polarization of the radiation, and a detailed analysis leads to the equation:

$$q = \frac{S_F(3 + p_F)\,f(\lambda_0)}{S_S(3 + p_s)\,\overline{f(\Delta\lambda)}}. \qquad (9)$$

In this equation S_S and S_F are the slopes in the plot of the signal from the scattering and fluorescent solutions respectively against the optical density E when E tends to zero. p_s and p_F are the linear polarizations of the right-angle scattering and fluorescence respectively; $f(\lambda_0)$ and $\overline{f(\Delta\lambda)}$ are factors characterizing the response of the detector to a photon of scattered exciting light of wavelength λ_0 and the average response to a photon of fluorescent light respectively. Thus

$$\overline{f(\Delta\lambda)} = \int_{\Delta\lambda} f(\lambda)\,I(\lambda)\,d\lambda \Big/ \int_{\Delta\lambda} I(\lambda)\,d\lambda. \qquad (10$$

In the last equation $I(\lambda)$ is the intensity of the fluorescence emitted at wavelength λ. From a knowledge of the fluorescence spectrum and the spectral response of the photomultiplier, $\overline{f(\Delta\lambda)}$ may be easily computed by graphical integration. On the other hand if a solution of a fluorescent substance is used as a proportional photon counter, $f(\lambda_0)/\overline{f(\Delta\lambda)} = 1$. Both methods have been used in the present case. A 10^{-2}M solution of 1-dimethylaminonaphthalene-5-sulphonate was used as a proportional photon counter as already described. Glycogen solutions were used to scatter the 2537 Å radiation. The polarizations of the scattering and fluorescence were measured by means of a polarizer of fourteen quartz coverslips mounted at the Brewster angle. The experimental values were corrected for transmission of the unwanted component which was approximately 4 % (Conn & Eaton, 1954). Relative quantum yields may be determined from the areas under the emission bands. If $E(\lambda)$ is the energy received by the detector from an interval of wavelength $\Delta\lambda$, $E(\lambda)/\Delta\lambda$ is the energy emitted in unit wavelength interval about λ. If $n(\lambda)$ is the number of photons in the same interval,

$$\frac{E(\lambda)}{\Delta\lambda} = n(\lambda)\,\frac{hc}{\lambda}, \qquad (11)$$

with $F(\lambda)$ denoting the signal from the detector corrected for change in response with wavelength and for losses in the monochromator, the quantity $\lambda F(\lambda)$ is proportional to $n(\lambda)$ if $\Delta\lambda$ is kept constant throughout. The areas under the plot of $\lambda F(\lambda)$ against λ are proportional to the number of photons emitted and the ratio of two such areas obtained under *identical conditions of excitation* is the ratio of the quantum yields. The condition of constant $\Delta\lambda$ is simply fulfilled with a grating monochromator by keeping the slit width constant, and this is the reason for the choice of λ rather than ν in the present instance. The optical densities were measured by means of a Uvispek spectrophotometer. Of the substances used the amino acids were commercial samples several times recrystallized. The dimethylaminonaphthalene derivatives were prepared according to Fussgänger (1902); 1-aminonaphthalene-3:6:8-sulphonate was a technical product recrystallized three times from 10 % NaCl solution.

Precision and errors

The fluorescence-excitation spectra and fluorescence spectra could be reproduced to ±1 %, i.e. the area under the bands of two samples done under similar conditions would differ by 1 % on the average.

The precision of the quantum-yield determination is discussed elsewhere. In general, the reproducibility gives $\Delta q = \pm 0{\cdot}01$ for $q < 0{\cdot}20$ and $\Delta q = \pm 0{\cdot}02$ for $0{\cdot}20 < q < 0{\cdot}5$.

RESULTS

Fluorescence-excitation spectra

Figs. 4–6 show the fluorescence-excitation spectra of the three aromatic amino acids plotted with the corresponding absorption spectra. Close correspondence was obtained for all three substances, including the fine structural details in phenylalanine.

Fluorescence spectra

They are shown in Fig. 7. In tryptophan and phenylalanine the overlap of emission and absorption bands is negligible and $F(\lambda)$ has been plotted as giving directly the molecular spectrum. In tyrosine, where appreciable overlap of the bands occurs, for a 10^{-5} M solution in a cell of 5 mm. thickness, $(\beta-1)\simeq 0{\cdot}1$ and $q(\beta-1)\simeq 0{\cdot}02$. The values have been corrected for attenuation only and $F(\lambda)/\alpha(\lambda)\bar{l}$ has been plotted as the molecular spectrum. Table 1 gives the position of the maxima of emission and the half-width of the bands. From an inspection of the values it appears that in a mixture of these amino acids it will be possible in many cases to detect the contribution from each of them.

In contrast to the absorption spectrum and the fluorescence-excitation spectrum, the emission spectrum of phenylalanine gives no sign of the presence of fine structure, although the resolution of the monochromator and the band width used in the scanning of the spectrum would certainly be

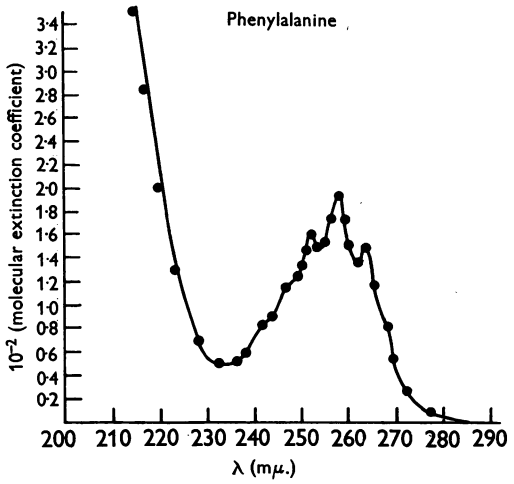

Fig. 4. Excitation spectrum of phenylalanine fluorescence in water. Abscissa: wavelength (mμ.). Ordinate: molecular extinction coefficient. The continuous line is the optical density spectrum; the dots are the values of log Ω in equation (5).

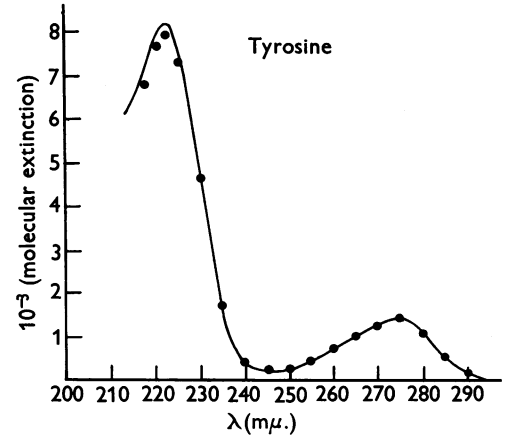

Fig. 5. Excitation spectrum of tyrosine fluorescence in water. Co-ordinates are as in Fig. 4.

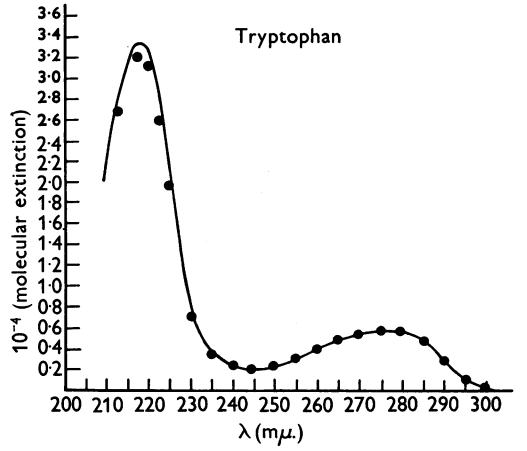

Fig. 6. Excitation spectrum of tryptophan fluorescence in water. Co-ordinates are as in Fig. 4.

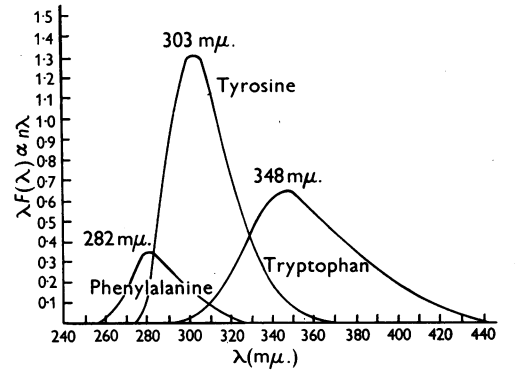

Fig. 7. Fluorescence spectra of the aromatic amino acids in water. Abscissa: wavelength (mμ.). Ordinate: relative number of quanta.

Table 1. *Maxima and band widths of the fluorescence of the aromatic amino acids in neutral aqueous solution*

λ_M is the emission maximum, $\lambda_{\frac{1}{2}}$ and $\lambda_{-\frac{1}{2}}$ are the two values of λ at which the emission becomes one-half of that at the maximum. All values are in mμ.

	λ_M	$\lambda_{\frac{1}{2}}$	$\lambda_{-\frac{1}{2}}$
Phenylalanine	282	298	270
Tyrosine	303	321	287
Tryptophan	348	383	323

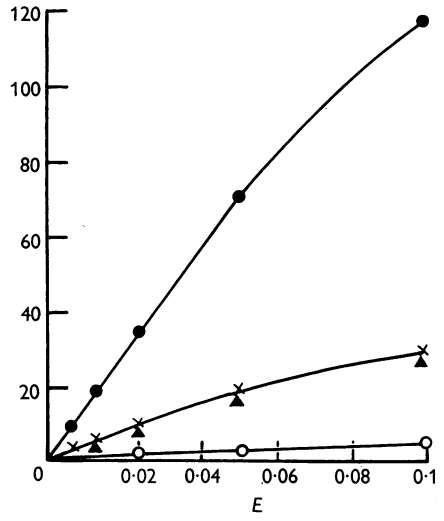

Fig. 8. Quantum yield of the aromatic amino acids in water. Integrating screen: 10^{-2}M solution of sodium 1-dimethylaminonaphthalene-5-sulphonate. Abscissa: optical density of solutions. Ordinate: signal from photomultiplier. ●, Glycogen; ×, tyrosine; ▲, tryptophan; ○, phenylalanine.

Table 2. *Quantum yield of neutral aqueous solutions of the aromatic amino acids*

q_1 is the quantum yield obtained by the use of a concentrated solution of 1-dimethylaminonaphthalene-5-sulphonate as an integrating screen, q_2 is the quantum yield obtained by using the direct response of the photomultiplier and equation 10. The value of $\overline{f(\Delta\lambda)}/f(\lambda_0)$ calculated from the fluorescence spectra and the change of detector response with wavelength is given in the third column. q_r is the yield relative to tyrosine as measured by the area under the emission band corrected for grating transmission. The relative grating transmission factors g calculated are shown in the last column.

	q_1	q_2	$\overline{f(\Delta\lambda)}/f(\lambda_0)$	q_r	g
Phenylalanine	0·045	0·038	0·97	0·23	0·95
Tyrosine	0·21	0·21	0·93	1	0·99
Tryptophan	0·19	0·20	0·76	0·92	0·887

capable of revealing it. This disappearance of fine structure in the fluorescence spectrum indicates the increased interaction between the excited molecular oscillator and the permanent water dipoles nearby. The fine structure of the phenylalanine emission spectrum may conceivably be found in polypeptides and proteins where the water dipoles in the vicinity of the amino acid are replaced by other entities. Fig. 7 also shows that approximately 15% of the quanta from tryptophan are emitted at wavelengths longer than 395 mμ., appearing as a violet fluorescence to the unaided eye.

Quantum yields

Fig. 8 shows the signal from the detector plotted against the optical density E of the solutions at 2537 Å, for solutions of the three amino acids in water (pH 6·5–7) and for glycogen solutions. The polarizations of the fluorescence for the three amino acids in water are less than 1/100, whereas the polarization of the 2537 Å radiation scattered from glycogens was found to be $0{\cdot}85 \pm 0{\cdot}01$. Therefore $3+p_F/3+p_s = 0{\cdot}78$ for the three amino acids. Most of the depolarization of the scattered light from glycogen is probably due to divergence of the exciting beam, which was uncollimated. The values of q calculated are shown in Table 2.

The *relative* efficiencies of the aromatic amino acids have also been measured by integration of the bands obtained in plotting the energy response of the detector against wavelength for constant wavelength band width as shown in Fig. 7. The relative values obtained by integration are in agreement with the absolute values obtained by the previous method, as shown in Table 2.

DISCUSSION

The preceding results show that the fluorescence observed in solutions of the aromatic amino acids is due to the amino acids themselves and not to accidental impurities present in the solutions.

In a recent publication of Shore & Pardee (1956) data are presented on the ultraviolet fluorescence of the aromatic amino acids which are at variance with those presented in this paper. The absolute fluorescence efficiencies quoted by these authors are two to three times lower than those given in Table 1. The relative efficiencies are also different, tyrosine being given as having only two-thirds of the tryptophan fluorescence. Moreover, a variation of the fluorescent efficiency with exciting wavelength, of up to 100% in one case, is claimed. We believe that these results are due to the method employed by Shore & Pardee for the determination of the fluorescent efficiencies. Their method depends on the comparison of the response of the photomultiplier to the exciting light on one hand and to

the fluorescence set up by the absorption of a known fraction of the exciting light on the other, the fluorescent and exciting light being separated by filters. Thus corrections for the geometry, integrated transmission of the filter and integrated response of the photomultiplier have to be calculated for each fluorescent substance. Although by methods neither fully described nor referred to in the paper the first two corrections have been estimated to one significant figure and the third calculated from technical data to the same accuracy, the authors give some of their results to three significant figures. Although the absolute values obtained under these conditions can be taken only as a very rough approximation, their relative figures are explained by the use of a filter with a cut-off at 300 mμ. to separate excitation from emission. Fig. 7 shows that such a filter will intercept about 80 % of the phenylalanine fluorescence, 40 % of the tyrosine and a negligible fraction of the tryptophan fluorescence. An overall transmission factor of 0·6 was used by Shore & Pardee for the three amino acids. The change in fluorescent efficiency with wavelength must be attributed to systematic errors in the method used, since the much more accurate technique of the excitation spectra gives no indication of such changes.

The overlap of the emission spectrum of tyrosine with its own absorption band is of the order of that found in fluorescein and rhodamine B, where transfer of the electronic energy from an excited molecule to a nearby molecule in the ground state is known to occur and to lead to the depolarization of the fluorescence observed in concentrated solutions (Gaviola & Pringsheim, 1924; Pheofilov & Sveshnikoff, 1941). From such depolarization measurements the distance at which probability of transfer equals probability of emission for parallel oscillators has been found for several dyes to be about 30 Å (Weber, 1954). From the number of tyrosine residues and the dimensions of ordinary globular protein molecules it appears that internal transfer of the excitation energy of the tyrosine must occur. Still more probable appears the transfer from phenylalanine to tryptophan or to tyrosine, from tyrosine to tryptophan and from all the aromatic amino acids to the haem group of haem–proteins. This is no doubt the reason for the high quantum yield of the photodissociation of carboxymyoglobin when illuminated by light absorbed by the aromatic residues of the protein (Bücher & Kaspers, 1946). Such possibilities of transfer place definite limitations on the interpretation of the action spectra of photochemical effects in the complex particulate systems of the living cell.

SUMMARY

1. The fluorescence-excitation spectrum, fluorescence spectrum and quantum yield are proposed as criteria to characterize a molecular species in solution as a fluorescent entity.

2. The fluorescence spectra of phenylalanine, tyrosine and tryptophan in neutral water solution are shown to consist of single bands in the ultraviolet with maxima at 282, 303 and 348 mμ. respectively.

3. The fluorescence-excitation spectra determined from 200 to 320 mμ. correspond accurately to the known absorption spectra of the amino acids, showing the constancy of the quantum yield over the range of wavelengths investigated.

4. The quantum yields ($\pm$ 1 %) in neutral water solutions are 4 % for phenylalanine, 21 % for tyrosine and 20 % for tryptophan.

5. The possibility of electronic energy transfer among the aromatic residues in proteins and to the haem in haemoproteins is discussed.

The financial help of the Medical Research Council is gratefully acknowledged. One of us (F.W.J.T.) is indebted to the Agricultural Research Council for a personal grant.

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